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# Antidiabetic and hypolipidaemic activities of methanolic extract of *Polyalthia longifolia* leaf var pendular (Annonaceae) in Streptozotocin-induced type II diabetic rats

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ABSTRACT: Extracts of *Polyalthia longifolia leaf var pendular (Annonaceae) Sonn* is used in herbal medicine by the Traditional Medicine Practitioners (TMPs) in Ilorin; Kwara State. Phytochemical screening, antidiabetic and hypolipidaemic effects of methanolic extract of *Polyalthia longifolia var pendular* leaf (MEPL) was evaluated in Streptozotocin (STZ) induced type II diabetic rats.

Type II diabetes mellitus was induced in overnight fasted wistar albino rats of both sex weighing 80-150g, by a single intraperitoneal (i.p) injection of 70mg/kg STZ in citrate buffer (pH 4.5), 15 minutes after intraperitoneal administration of 110mg/kg Nicotinamide in normal saline.

Methanolic extract of the plant was administered orally at doses of 100, 200, 400 and 800 mg/kg body weight for 21 consecutive days. Fasting Blood Glucose (FBG) was estimated on overnight fasted rats on day 0, 4, 7, 14 and 21.The diabetic rats were sacrificed on day 21 and their lipid profile determined. The extracts of *Polyalthia longifolia var pendular leaf* produced dose-dependent blood glucose lowering activity. There was however, no significant difference between the FBG of the diabetic rats treated standard drug Glibenclamide and those treated with the plant extract ($p>0.05$).

Methanolic extract of *Polyalthia longifolia* at dose of 400mg/kg body weight significantly reduced $P<0.05$ elevated serum total cholesterol (50.78%), triglycerides (30.76%), high density lipoprotein (60.00%) and low density lipoprotein (50.86%).

Phytochemical screening of *Polyalthia longifolia* leaves revealed the presence of tannin, saponin , cardiac glycoside ,terpenoids, alkaloids and flavoniods. It also contains the following metals:

Zn (0.68±0.01mg/100g), Cu (0.51±0.01mg/100g) , Ni (0.31±0.04mg/100g), Pb (0.57±0.1mg/100g), Fe (112.83±0.13mg/100g), P (2.45 ± 0.01mg/100g S (1.22±0.01mg/100g) Methanolic extract of *Polyalthia longifolia var pendular leaf* have both antidiabetic and hypolipidaemic properties to justify its use in herbal medicine by TMPs in Ilorin, Kwara State for the treatment of diabetes mellitus.

**Keywords**: Antidiabetic activities, Hypolipidaemia, Streptozotocin, Glibenclamide, Phytochemical screening, Traditional Medicine.

## Introduction

Diabetes mellitus (DM) is a chronic metabolic disease in which the blood sugar levels are elevated. It is caused by the inability of tissues to carryout normal metabolism of carbohydrate, fats, and proteins due to absolute or relative lack of insulin. Gilman *et al* (1996).This syndrome is characterized by hyperglycaemia,, glucosuria, polyuria ,hyperlipidaemia and loss of weight. De Sereday *et al* (2004).

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Before the introduction of Insulin, management of Diabetes mellitus relied on dietary measures which included the use of traditional plant therapies. Maiti *et al* (2008).Therefore, many plants with ethno -pharmacological uses are still being employed by traditional medicine practitioners who are widely consulted by people at the grassroots. Bailey,C.T and Day,C(1989) .In -view of this ,the World Health Organization has recommended that traditional plants treatment for diabetes needs further evaluation. WHO (1980) and WHO (1994) *Polyalthia longifolia var pendular (Annonaceae)* is an evergreen plant ,native of India and well distributed in tropical countries of the World, West Africa and Nigeria. It is commonly planted as ornamental plant and over 30 feet in height. Chen *et al* (2000).

*P. longifolia* is known locally as Masqurade tree,Asoka and Agunmadorun (Yoruba).Its ethno-botanical uses include treatment of fever, inflammatory pain and hypertension. Saleem *et al* (2005). Bioactive Phytochemical isolates of this plant include terpenes, lactones, indolosesquiterpenes and diterpenes .These isolates have been shown to have bioactivity against cancer cell lines, Human immunodeficiency virus and antimicrobials..Verma *et al*(2008), Saeptou *et al* (2010) , Nair R and Chanda S (2006). It was observed that leaves of *Polyalthia longifolia* is also prescribed as herbal recipe by traditional medicine practitioners (TMP) in Ilorin, Kwara State for the treatment of Diabetes mellitus, malarial fever , cough and hypertension. This study therefore aims at investigating the antidiabetic and antilipidaemic activities of this plant on type II model of DM to justify their use in traditional medicine.

## Materials and Methods

### Collection and Processing of Plant Material

*P. longifolia* leaves (Annonaceae) was collected from the premises of University of Ilorin Teaching Hospital (Temporary site), Ilorin. The plant was taxonomically identified and authenticated by Professor F.A .Oladele of Plant Science Department, University of Ilorin. The plant with voucher number UIH/872 was deposited at University of Ilorin herbarium. The leaves were air dried for 8 weeks and pulverised into fine powder using clean Mortar and Blender.

### Preparation of Extract

One hundred grams of the powdered leaves of *P.longifolia* was macerated with 1L of absolute methanol (BDH) and allowed to extract at room temperature for 72h.The plant was filtered and the filtrate concentrated with rotary evaporator attached to a vacuum pump. The concentrated extract was then freeze-dried at Central Science Laboratory, Obafemi Awolowo University, Ile-Ife; Osun State.
The freeze- dried powdered methanolic extract Polyalthia longifolia leaves (MEPLL) was reconstituted in 0.9% normal saline at various concentrations and used for the assay.

### Phytochemical Screening

Qualitative and quantitative screening of methanolic extract of *P.longifolia* leaves was carried out by methods described by Harbone (1982), Sofowora (1986) and Edeoga *et al* (2005) and Okwu and Josiah (2006)

### Antidiabetic Studies

### Standard drugs

Streptozotocin ( STZ) and Nicotinamide used for the Induction of diabetes were procured from Sigma-Aldrich (Netherlands).Glibenclamide (Daonil was purchased from One-Step Pharmacy Shop, Saw-Mill IIorin, Kwara State.

### Animals

Thirty in-bred Wistar albino rats (80-150g) of either sex from Pharmacology animal house, University of Ilorin were selected for this experiment. The animals were kept under standard conditions of 12:12 h light and dark cycle

in propylene cages and fed with standard animal feed (Bendel Feeds) and water *ad libitun* .The animals were acclimatised to laboratory conditions for seven days before the commencement of the induction process.

**Antihyperglycaemic Studies**

Non-insulin dependent diabetes mellitus (NIDDM) or type II diabetes mellitus was induced in overnight fasted rats of both sexes weighing (80-150g) by methods described by Maiti *et al* (2008) and Masiello *et al* (1998) with slight modifications.

A single intraperitoneal (i.p) injection of 70mg/kg body weight of STZ in Citrate buffer ($P^H$ 4.5) 15 minutes after i.p administration of 110mg/kg body weight of Nicotinamide in normal saline.

Fasting Blood Glucose (FBG) and weights of each group of rats pre- induction and post treatment were determined. Diabetes mellitus was confirmed in the rats by elevated fasting blood glucose (hyperglycaemia) 48h post induction. The rats were stabilized for 3 days and those found with permanent NIDDM **(FBG>12mmol/L or FBG>200mg/dl)** were used for the antidiabetic study.

**Experimental Design**

Eighteen diabetic rats (80-150g) were randomly assigned into six groups of three rats each. Groups I and V normal and positive control rats received 10ml/kg body weights normal saline orally (Vehicle only); while Groups II-IV test rats were orally treated with 200mg/kg,400mg/kg and 800mg/kg body weights of MEPLL extracts respectively. Group VI rats were treated with 0.25mg/kg Glibenclamide (Daonil) orally.

MEPLL extracts were administered to the rats daily for 7 days. Fasting blood sugar was determined in overnight fasted rats using One-Touch Glucometer on day 0,2,7,14 and 21 post induction. Haematological parameters and Lipid profiles were measured after the animals were sacrificed on day 21 under Chloroform anaesthesia.

**Statistical Analysis**

The results obtained were either expressed as Mean ±SD or Mean±SEM and analysed for statistical significance using student t-test computerised Primer Biostatistics software.

## Results

**Table 1: Phytochemicals present methanolic extracts of *Polyalthia longifolia* leaves.**

| Secondary Metabolites | *X. aethiopica* |
|---|---|
| Saponin | Positive |
| Cardiac glycoside | positive |
| Alkaloids | positive |
| Tannin | positive |
| Volatile Oil | negative |
| Flavoniods | positive |
| Terpenoids | Positive |

**Table 2: Trace metal content of *Polyalthia longifolia leaves***

| Elements | Concentration mg/100g* |
|---|---|
| Mn | Nd |
| Zn | 0.68±0.01 |
| Cu | 0.51±0.01 |
| Co | Nd |
| Cd | Nd |
| Ni | 0.31±0.01 |
| Fe | 112.83±0.14 |
| Pb | 0.57±0.01 |

*The values represent Mean ± SD (N =3)

Table 3: Phosphorus and Sulphur content of *Polyalthia longifolia leaves*

| Element | Concentration mg/100g* |
|---|---|
| P | 2.45±0.01 |
| S | 1.22±0.01 |

*The values represent Mean ±SD (N=3)

Table 4: Secondary Metabolites in *Polyalthia longifolia* leaves.

| Secondary Metabolites | Concentrations in mg/100g |
|---|---|
| Saponin | 238.87±0.09 |
| Alkaloids | 160.56±0,02 |
| Tannin | 0.66 ± 0.01 |
| Flavonoids | 0.17 ±0.01 |

**Plate1 :PICTORIAL VIEW OF POLYALTHIA PLATE PENDULA LEAVES**

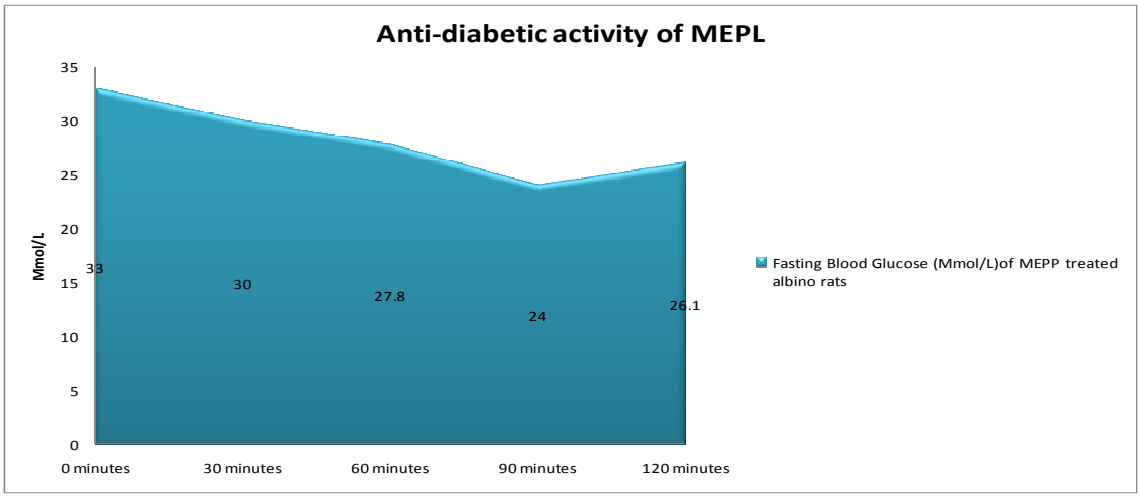



**Figure 1:PRELIMINARY IN-VIVO PHARMACOKINETICS OF ANTI DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF POLYALTHIA LONGIFOLIA LEAVES (MEPLL)**

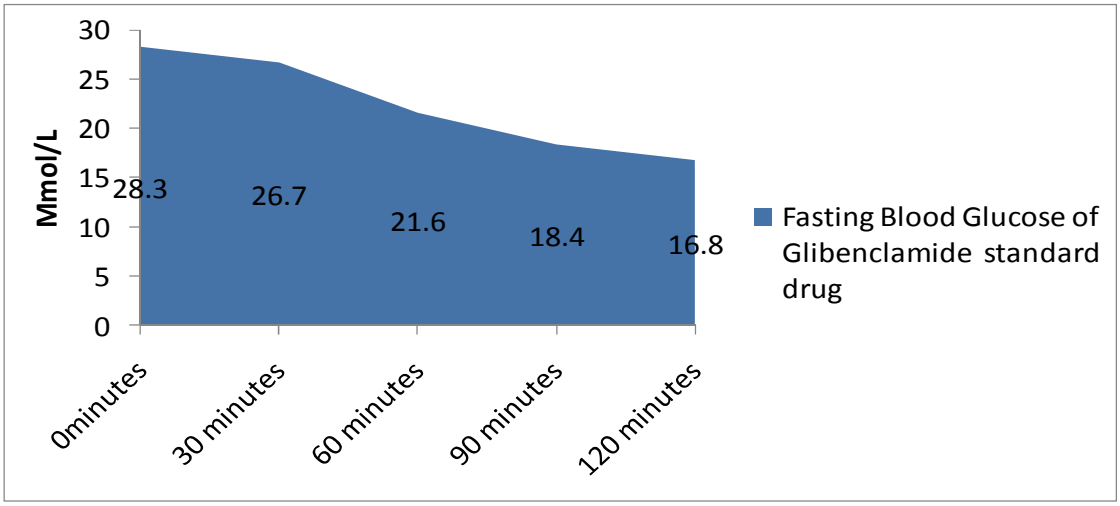
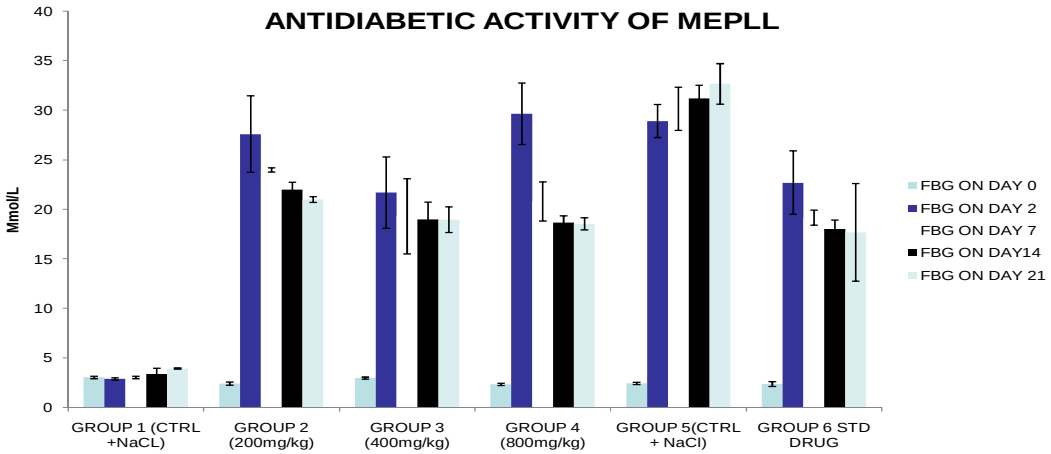



**Figure 2:PRELIMINARY IN-VIVO PHARMACOKINETICS OF ANTI DIABETIC ACTIVITY OF GLIBENCLAMIDE (DAONIL) STANDARD**

Table 4: Antidiabetic activity of defatted methanolic extract of *Polyalthia longifolia* leaves (MEPLL)

| Treatment | Day 0 | Day 2 | Day 7 | Day 14 | Day 21 |
|---|---|---|---|---|---|
| | FBG (mmol/l) | FBG (mmol/l) | FBG (mmol/l) | FBG (mmol/l) | FBG (mmol/l) |
| Group 1 (Non-diabetic + Normal saline) | 3.03 ± 0.12 | 2.87 ± 0.12 | 3.03 ± 0.12* | 3.37 ± 0.59* | 3.93 ± 0.05* |
| Group II (Diabetic + 200 mg MEPL) | 2.40 ± 0.16 | 27.6 ± 3.86 | 24.0 ± 0.21 | 22.0 ± 0.75 | 21.0 ± 0.30 |
| Group III (Diabetic + 400 mg MEPL) | 2.97 ± 0.10 | 21.7 ± 3.61 | 19.3 ± 3.8 | 19.0 ± 1.73 | 18.96 ± 1.30 |
| Group IV (Diabetic + 800 mg MEPL) | 2.33 ± 0.12 | 29.7 ± 3.10 | 20.8 ± 1.97** | 18.7 ± 0.68** | 18.53 ± 0.61** |
| Group V (Diabetic + Normal saline) | 2.43 ± 0.12 | 28.9 ± 1.68 | 30.2 ± 2.18* | 31.2 ± 1.34* | 32.67 ± 2.05* |
| Group VI (Diabetic + 0.25 mg/kg Glibenclamide) | 2.36 ± 0.23 | 22.7 ± 3.20 | 19.2 ± 0.76*** | 18.0 ± 0.90*** | 17.67 ± 4.93*** |

Each value represents the mean ± SEM.
*P<0.01 Normoglycaemic control rats vs Diabetic untreated control rats (S).
**P<0.05 MEPL treated diabetic rats vs Untreated diabetic control (S).
***P>0.68 Standard drug treated diabetic rats vs MEPL treated diabetic rats (NS)
Student's t-test Primer Biostatistics Software.

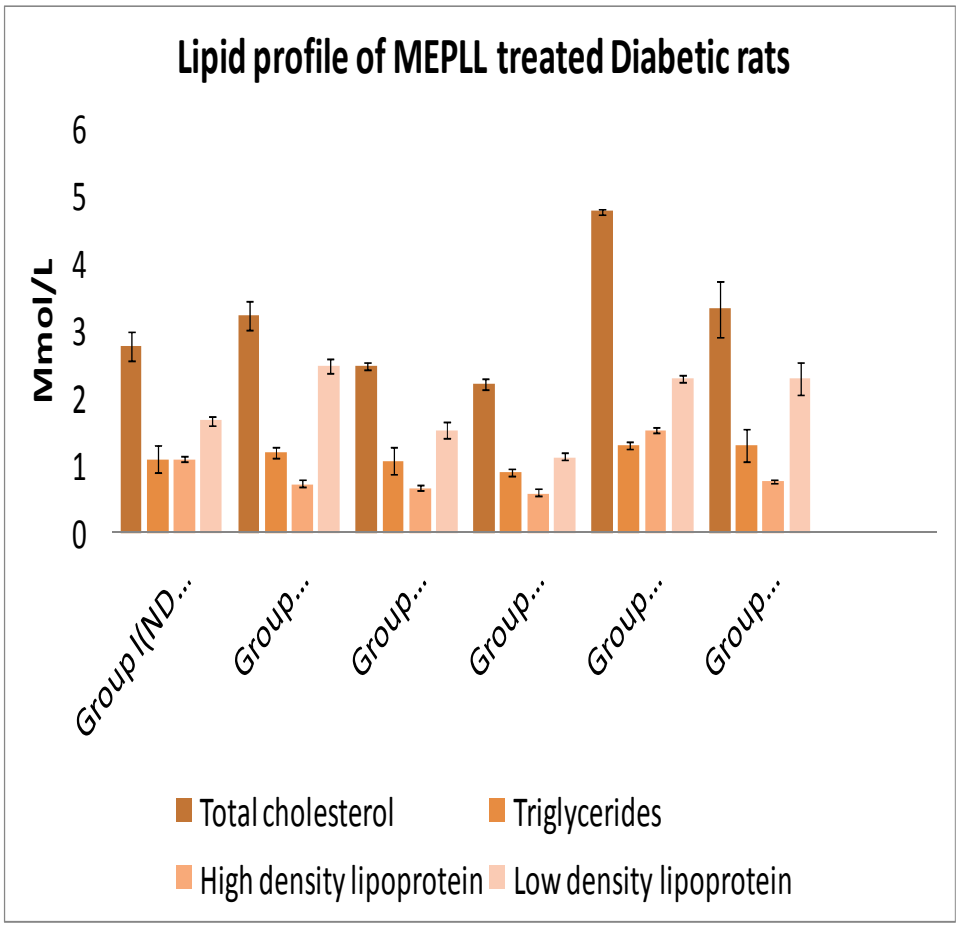



FIQURE 3:ANTIDIABETIC ACTIVITY OF DEFATTED METHANOLIC EXTRACT OF POLYALTHIA LONGIFOLIA (MEPLL)*

Table 5: Hypolipidaemic activity of methanolic extract of *Polyalthia longifolia* leaves (MEPLL)

| Treatment | Total cholesterol | Triglycerides | High Density Lipoproteins | Low Density Lipoproteins |
|---|---|---|---|---|
| | (mmol/l) | (mmol/l) | (mmol/l) | (mmol/l) |
| Group 1 (Non-diabetic + Normal saline) | 2.77 ± 0.21* | 1.10 ± 0.21* | 1.10 ± 0.04* | 1.67 ± 0.07* |
| Group II (Diabetic + 200 mg MEPL) | 3.23 ± 0.22 | 1.20 ± 0.08 | 0.73 ± 0.05 | 2.47 ± 0.11 |
| Group III (Diabetic + 400 mg MEPL) | 2.47 ± 0.05 | 1.07 ± 0.20 | 0.67 ± 0.03 | 1.53 ± 0.12 |
| Group IV (Diabetic + 800 mg MEPL) | 2.20 ± 0.08** | 0.90 ± 0.05** | 0.60 ± 0.05** | 1.13 ± 0.05** |
| Group V (Diabetic + Normal saline) | 4.77 ± 0.03* | 1.30 ± 0.05* | 1.53 ± 0.03* | 2.30 ± 0.05* |
| Group VI (Diabetic + 0.25 mg/kg Glibenclamide) | 3.33 ± 0.42*** | 1.30 ± 0.24*** | 0.77 ± 0.03*** | 2.30 ± 0.24*** |

**Each value is Mean ± SEM.**
***P<0.01 Normoglycaemic control rats Vs Diabetic untreated control rats (S)**
****P< 0.01 MEPL treated diabetic rats Vs Untreated diabetic control : TC(50.78%),TG(30.76%),HDL(60%) and LDL(50.86%)**
*****P < 0.01 Standard drug control Vs untreated diabetic rats(S)**
**(STUDENT'S T-TEST PRIMER BIOSTATISTIC SOFTWARE)**




Fiigure 4:HYPOLIPIDAEMIC ACTIVITY MEPLL IN DIABETC ALBINO RATS

## Discussion

*Polyalthia longifolia var pendular* (Plate 1) is ethno-botanically used as herbal recipe by the traditional medicine practitioners (TMP) in Ilorin Kwara State for the treatment of malarial fever, Inflammatory pain, hypertension and diabetes mellitus. The TMPs do not have scientific knowledge on the therapeutic efficacy and toxicology of this plant, since their herbal knowledge is passed orally through their ancestors from generations to generation Bubayero (1986).

Phytochemicals present as mineral elements and secondary metabolites play vital roles in the bioactivity exhibited by medicinal plants used ethno -botanically. Kar and Choudhary (1994). Phytochemical analysis of *Polyalthia longifolia leaves* showed the presence of saponin, tannin, cardiac glycosides, flavoniods and alkaloids.(Table 2).

Trace elements are required in small quantity in diet to serve various purposes in human physiology and metabolism. Table 2 showed the trace metal, Phosphorus and Sulphur contents of *Polyalthia longifolia* leaves.
These elements, though in small quantity serve as co-factor in the synthesis and metabolism of body enzymes, haemoglobin, vitamin $B_{12}$ and thyroxin, while their deficiencies could lead to diseases and death. Essential minerals such as Ca, Zn, K, Mn and Cr have been shown to be associated with the mechanisms of insulin release and its activity in different animals and in human beings, Kar *et al* (1999), Mertz(1981),Underwood and Mertz(1986) and Schroeder(1996).

The preliminary hypoglycaemic activity of methanolic extract of *Polyalthia longifolia* leaves (MEPLL) at a dose of 100mg/kg was demonstrated in diabetic albino rats in 60 min and reached its minimal value of 24mmol/L from 30mmol/lLin 90 min.(Figs 1 and 2).
A single high dose of Streptozotocin (STZ) injection can produce type I diabetes by destroying the B-cells of the pancreas in albino rats but a mild dose of STZ can induce type II diabetes in adult rats. Maiti *et al* (2005). A new model of type II diabetes mellitus has been produced by administering a combination of STZ and Nicotinamide .Masiello *et al* (1998)

Fasting blood glucose level in diabetic rats is a good index for monitoring diabetes. The extracts of MEPLL produced dose-dependent blood glucose lowering activity (Table 4 ). There was however, no significant difference between the FBG of the diabetic rats treated standard drug Glibenclamide and those treated with the plant extract $p>0.05$ (Figure 3 and table 4). The hypoglycaemic activity of MEPLL in this research also agrees with the findings of Nair *et al* (2007) that extracts of Polyalthia longifolia produced glucose lowering activity in alloxan-induced diabetic rats. The significant decrease in the levels of fasting blood glucose in MEPLL diabetic rats may be due to cytoprotective activity of the extract on diabetogenic activity of STZ. MEPLL contain antioxidant phytochemicals: flavoniods and saponin which can scavenge and protect the pancreas against degenerative activities of the free radicals.(Tables 1 and 2).

Mi-Kyung *et al* (2005) and Adewole *et al* (2006) have also reported the ctyto- protective activity flavoniods (quercerin, melatonin) from plants in reducing oxidative stress and injury associated with STZ-induced diabetic mellitus thereby enhancing further synthesis of insulin by the pancreatic B-cells..

Diabetes is associated with hyperlipidemia. Maiti *et al* (2005). Methanolic extract of MEPLL at a dose of 400mg/kg body weight significantly reduced $P<0.05$ elevated serum total cholesterol (50.78%), triglycerides (30.76%), high density lipoprotein (60.00%) and low density lipoprotein (50.86%).(Table 5 and Figure 4) .The serum total cholesterol ,triglycerides, high density lipoproteins and low density lipoproteins have been decreased significantly in type II diabetic rats after treatment with methanolic extract of Polyalthia longifolia. This effect observed may be due to low activity of cholesterol biosynthesis enzymes or low level of lipolysis which are under the control of insulin. Sharma *et al* (2003).

The observed hypolipidaemic activity of MEPLL in this study is at variance with the reported work of Nair *et al* (2007) ; who observed that *Polyalthia pendula* extract and powder did not modify biochemical properties significantly in alloxan diabetic rats. Medicinal bioactivities of plants are known to show variation due to bio-diversity, geographical and environmental differences, and insensitive animal model. This may explain the variation observed. Kar *et al* (2003). Thus methanolic extract of *Polyalthia longifolia var pendular leaves* have both antidiabetic and hypolipidaemic properties to justify its use in herbal medicine by traditional medicine practitioners, in Ilorin, Kwara State; Nigeria for the treatment of diabetes mellitus.

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